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The microtubule associated protein syntabulin is required for glucose-stimulated and cAMP-potentiated insulin secretion

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ABSTRACT

Syntabulin is a microtubule-associated protein that mediates anterograde transport of vesicles to neuronal processes. Here, we found that syntabulin was expressed in mouse pancreas and insulin-secreting β-cells, and that it partially co-localized with microtubule and insulin-containing granules. The association of syntabulin with these organelles increased upon glucose stimulation. Knock-down of syntabulin by shRNA reduced both basal and glucose-stimulated insulin secretion, and diminished cAMP-Epac2 and cAMP-PKA potentiated insulin secretion. Additionally, syntabulin was preferentially phosphorylated by the Epac2 agonist 8-pCPT-2′-*O*-Me-cAMP, suggesting that syntabulin could be a novel effector of Epac2 and play a critical role in cAMP-enhanced insulin secretion.

1. Introduction

Glucose-stimulated insulin secretion involves both fusion at the plasma membrane of insulin-containing granules of a readily reliable pool (RRP) and the mobilization and trafficking of insulin-containing granules to the cell surface from intracellular reserve pools [1,2]. These events are triggered by increased glucose flux into β-cells, resulting in increased glycolysis and an elevation in the intracellular ATP/ADP ratio and leading to the closure of K_{ATP}-channels and depolarizing cell membrane, opening voltage-gated calcium channels, and increased cytosolic calcium concentration [3–5]. It is well established that this increase in intracellular calcium is a necessary trigger for insulin secretion and is thought to regulate both the initial exocytosis of primed granules and subsequent trafficking/mobilization of stored insulin granules [6]. Previous studies have disclosed that recruitment of insulin-containing granules to the plasma membrane driven by kinesins plays a critical role in sustained glucose-stimulated insulin secretion and involves granule transport along microtubules, thus important for the second phase of insulin secretion [7,8]. It is now known that microtubule-based granule transport involves both rapid and slow component of exocytosis [9,10], and therefore is required for both phases of glucose-induced insulin secretion [11]. Kinesins are microtubule-dependent ATPase that use ATP hydrolysis to move cargoes along microtubules [12]. It has been found that kinesin is required for axonal transport in neuronal cells [13] and recruits granules to the release sites of Ca^{2+}-regulated exocytosis in sea urchin embryos [14]. Three conventional kinesin genes (*Kif5a*, *Kif5b* and *Kif5c*) have been identified [8]. Among which *Kif5b* is expressed in primary mouse pancreatic β-cells [15] and involved in trafficking and exocytosis of insulin-containing granules [8].

Syntabulin is a kinesin-1 family membrane 5B (Kif5b) adaptor protein and a microtubule-associated protein that mediates anterograde transport of syntaxin-1 to neuronal processes [16]. It has been found that syntabulin functions as a linker molecule by attaching the cargo vesicles to conventional kinesin I and mediating the transport of cargo vesicles to the neuronal processes [16]. In live hippocampal neurons syntabulin was found to co-localize and co-migrate with GFP-Bassoon-labeled vesicles alone axonal processes [17]. Knock-down of syntabulin results in the impaired trafficking of synaptic vesicles to nerve terminals and thus diminishes synaptic transmission [17]. Moreover, expression of syntabulin dominant-negative mutants or suppression of syntabulin

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expression inhibits the activity-induced recruitment of GFP-Bassoon at release sites [17]. All these studies indicate that syntabulin functions as a linker molecule of vesicles and microtubules, and is essential for vesicle trafficking and exocytosis. In this study we for the first time show that syntabulin is also present in pancreas and insulin-secreting β-cells. As its role for maintenance of neurotransmission, syntabulin is essential for glucose-stimulated insulin secretion and cAMP-potentiated insulin secretion.

2. Materials and methods

2.1. Animal experiments

Female C57BL/6 mice (4-6w) were purchased from the Experimental Animal Centre of Guangdong Academy of Medical Science (Guangzhou, China). The mice were killed by cervical dislocation. Tissues from brain and pancreas were isolated for Western blot analysis. All animal procedures were carried out according to the Principles of Laboratory Animal Care and approved by the Shenzhen University Animal Care Committee.

2.2. Cell lines and cell culture

MIN6 cells were kindly provided by Professor Xiaoying Li from Shanghai Institute of Endocrine and Metabolic Diseases. MIN6 cells (passages 31–42) were grown in Dulbecco's modified eagle medium (DMEM) containing 25 mM glucose supplemented with 15% (w/v) heat inactivated fetal bovine serum, 10 mM HEPES buffer, 50 μM beta-mercaptoethanol (Sigma Aldrich), 100 μM penicillin G and 100 mg/ml streptomycin [18]. INS-1E cells were kindly provided by Professor Yong Liu from Shanghai Institue for Nuitritional Sciences of Chinese Academy of Sciences. INS-1E cells (passages 48–60) were grown in RPMI1640 medium supplemented with 10% (w/v) fetal bovine serum, 10 mM HEPES buffer, 1 mM sodium pyruvate, 50 μM beta-mercaptoethanol, 100 μM penicillin G and 100 mg/ml streptomycin. All cells were constantly kept at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

2.3. RT-PCR

Total RNA from INS-1E cells was isolated using TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. One microgram of total RNA was reverse transcribed to single-stranded cDNA using random hexamer primers and Superscript II (Invitrogen). The cDNA from the reverse transcription reactions was used for amplification using the following protocol: 2 min at 95 °C, 32 cycles of 30 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C. Primers used were human-syntabulin-F: 5′-ATG GTG ACT GCC ACC ACC ACA; human-syntabulin-R: 5′-TTA GGT TTT CAT GTG GAA AGC; human-insulin-F: 5′-ATG GCC CTG TGG ATG CGC CTC; human-insulin-R: 5′-CTA GTT GCA GTA GTT CTC CAG. PCR products were analyzed on a 2% agarose gel and confirmed by sequencing.

2.4. Western blot

A 10 mm tissue segment or cell pellets were incubated in lysis buffer (RIPA, BioTeke) supplemented with 1 mM protease inhibitor cocktail (CALBIOCHEM) for 30 min on ice, followed by centrifugation at 14,000 rpm for 15 min at 4 °C. Equivalent protein amounts were resolved using SDS–PAGE gels and transferred onto a polyvinylidene difluoride (PVDF) membrane by electrophoresis. The membranes were immunoblotted with the following primary antibodies: polyclonal rat-anti syntabulin (Santa Cruze Biotechnology, 1:1500); polyclonal rat anti-phosphorylated syntabulin (1:500) and polyclonal rat anti-β-actin (Santa Cruz Biotechnology, 1:1000,) overnight at 4 °C, followed by incubation with a horseradish-peroxidase-conjugated goat anti-rabbit secondary antibody (Cell Signaling, 1:1000) at room temperature for 1 h. Immunoreactive bands were revealed by enhanced chemiluminescence (SuperSignal® West Pico Chemiluminescent Substrate kits, Thermo Scientific) and visualized by the KODAK Image Station 4000MM PRO imaging system and software according to the manufacture's instruction. Band intensities were quantified by scanning densitometry (Gel-Doc2000, Bio-Rad), analyzed with Quantity One™ (Bio-Rad) and normalized against the level of β-actin.

2.5. Indirect immunofluorescence and confocal microscopy

INS-1E cells cultured on glass coverslips were fixed with 100% ice-cold methanol for 10 min at −20 °C. After blocking unspecific binding by incubating for 30 min with 1% BSA, cells were incubated overnight at 4 °C in the presence of a rabbit anti-syntabulin antibody (1:100) and a mouse anti-α-tubulin antibody (Abcam, 1:100); a rabbit anti-insulin antibody (Cell Signaling, 1:100) and a mouse anti-α-tubulin antibody (1:100); or a rabbit anti-syntabulin antibody (1:100) and a mouse anti-insulin antibody (1:100), respectively. Cells were then incubated at 37 °C for 1 h with Alexa-Fluor488-labeled goat anti-mouse (Invitrogen, 1:100) and Alexa-Fluor546-labeled goat anti-rabbit (Invitrogen, 1:100) secondary antibodies. Images were obtained with an OLYMPUS FV1000 confocal laser-scanning microscope.

2.6. Immunoprecipitation

Two microliter of normal rabbit IgG and 2 μl of rabbit antibody specific to syntabulin (Santa Cruz) were incubated with Protein A/G beads with 300 μl TBST (0.1% Triton X-100) respectively overnight at 4 °C with gentle rotation. After three washes with TBST, cell lysates of different treated cells were added to each tube and incubated for 3 h at 4 °C. After extensive washing, proteins remaining bound to the beads were then analyzed by Western blot with monoclonal antibody specific to microtubule (Abcam, 1:2000).

2.7. Knock-down of syntabulin by RNA interference

Adenoviral short hairpin RNA for rat and mouse syntabulin pAd-syntabulin-shRNA (Syntabulin-shRNA) were constructed by inserting double-stranded oligo DNA with *EcoRI* and *BamHI* restriction enzyme sites (AATTCagacttgagctccatgatttcagaagctgtctgaaatcatggagctcaagtctaataaaG and GATCCtttattagacttgagctccatgatttcagacagcttctgaaatcatggagctcaagtctG) into the adenovirus vector with reporter gene GFP cut by the same enzymes. The plasmid constructs were confirmed by sequencing. 2×10^5 INS-1E cells were seeded in 12-well plates one day before transfection. Cells (~80% confluence) were then transfected with 1.6 μg Syntabulin-shRNA or vector control using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Seventy-two hours after transfection, the expression of syntabulin was analyzed by Western blot with β-actin as an internal control. The knock-down efficiency was quantified and normalized against the level of β-actin.

2.8. Measurements of insulin secretion

2×10^5 INS-1E cells were seeded in 12-well plates one day before transfection and then transfected with 1.6 μg Syntabulin-shRNA or vector control using Lipofecta mine 2000 (Invitrogen). Seventy-two hours after transfection, INS-1E cells were washed twice with KRB (containing in mM: 129 NaCl, 4.8 KCl, 1 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 5 $NaHCO_3$, 2.8 glucose, and 10 HEPES, supplemented with 0.1% BSA, pH 7.4) and preincubated in 1 ml KRB

buffer for 1 h at 37 °C. Subsequently, the medium was replaced by 1 ml KRB containing 2.8 mM glucose or 16.8 mM glucose alone, or in the presence of 10 μM forskolin (FSK)(Sigma), 100 μM 6-BNZ-cAMP-AM (6-BNZ) (Biolog Life Science), 1 μM 8-pCPT-2′-*O*-Me-cAMP (ESCA-AM) (Biolog Life Science) for 30 min, respectively. The supernatants were collected for measurements of secreted insulin and the attached cells were harvested for determination of total cellular protein content and protein expression. Insulin secretion was assayed by an Insulin (Rat) Ultrasensitive ELISA kit (ALPCO Diagnostics). Insulin levels (ng/ml) were normalized against total cellular protein content in mg and expressed as percentage change as compared with vector control.

2.9. Statistical analyses

All data were expressed as mean ± S.E.M. To test for statistical significance an unpaired Student's *t*-test was applied. Data were considered significant when $P < 0.05$.

3. Results

3.1. Syntabulin is expressed in mouse pancreas and insulin-secreting β-cells, and co-localized with microtubule and insulin-containing granules

Syntabulin is a microtubule associated protein and widely expressed in different tissues including brain, liver, heart, etc. [16]. Northern blotting results suggested that syntabulin was expressed in human pancreas (Su et al., unpublished data). We therefore tested whether syntabulin was also expressed in mouse pancreas using Western blot analysis. Results showed that syntabulin was not only expressed in mouse brain as reported previously [16], but also expressed in mouse pancreas (Fig. 1A). In addition syntabulin was readily detected in insulin-secreting INS-1E cells and MIN6 cells as determined by RT-PCR (Fig. 1B) and Western blot (Fig. 1C), respectively.

In hippocampal neurons syntabulin has been found to attach secretory vesicles to microtubules [16], we next determined the subcellular distribution of syntabulin in INS-1E cells. Confocal fluorescence images clearly showed that both syntabulin and insulin-containing granules were abundantly expressed in the cytoplasm of INS-1E cells and resided in multiple granulated foci. At un-stimulated conditions a few co-localizations of syntabulin (Fig. 2A; Suppl. Fig. 2) or insulin (Fig. 2B; Suppl. Fig. 2) with microtubule were observed. Accordingly some of syntabulin was co-localized with insulin-containing granules (Fig. 2C; Suppl. Fig. 2) in this case. By contrast, when INS-1E cells were stimulated with 16.8 mM glucose, co-localizations of syntabulin (Fig. 2D; Suppl. Fig. 3) or insulin (Fig. 2E; Suppl. Fig. 3) with microtubule were increased. The association of syntabulin with microtubule was further confirmed by immunoprecipitation assay (Fig.3). Interestingly upon 1 min (Suppl. Fig. 1C) or 10 min (Fig. 2F) of 16.8 mM glucose stimulation, syntabulin was readily detected to be co-localize with insulin-containing granules in the cytosol (Fig. 2F, Suppl. Fig. 1C), although many insulin-containing granules evidently resided at the plasma membrane. These data indicate that syntabulin may involve in the glucose-regulated microtubule-based translocation of insulin-containing granules.

3.2. Knock-down of syntabulin inhibits glucose-stimulated insulin secretion

Next we explored the importance of syntabulin for insulin secretion in β-cells. First, we adopted a short hairpin RNA (shRNA) approach to knockdown syntabulin in INS-1E cells. Transient transfection of INS-1E cells with adenoviral vectors coding for shRNA constructs against syntabulin resulted in decreased protein expression of syntabulin as determined by Western blot (Fig. 4A). Quantitative analysis of knock-down of syntabulin using shRNA revealed a 45% decrease in syntabulin protein level, as compared to vector control (Fig. 4B). The suppression efficiency using shRNA in our study is comparable to the previous reports in neurons [16,19]. Next, we assessed the effect of syntabulin downregulation on insulin secretion in response to a glucose challenge in INS-1E cells. In the syntabulin-downregulated cells insulin secretion in response to 2.8 mM or 16.8 mM glucose was reduced by 11% or 37%, respectively, in comparison to vector-transfected cells (Fig. 4C). These data suggest that syntabulin is required for both basal and glucose-stimulated insulin secretion.

3.3. Syntabulin is involved in cAMP-Epac2-potentiation of glucose-stimulated insulin secretion

Epac2 is the key isoform of Epac (exchange protein directly activated by cAMP) responsible for cAMP-regulated insulin secretion in pancreatic β-cells [20,21]. It has been found that Epac directly interacts with microtubules and mediates cAMP-regulated microtubule formation [22]. We therefore explored whether syntabulin would be an effector of cAMP-Epac2 in INS-1E cells. The level of syntabulin protein expression upon secretagogue stimulation was determined by Western blot. Application of 10 μM forskolin (a general stimulator of adenylyl cyclase) or 1 μM 8-pCPT-2′-*O*-Me-cAMP (a specific Epac2 agonist) produced a 1.72- or 1.70-fold increase ($P < 0.05$) in phosphorylated syntabulin level, respectively, as comparison to control (Fig. 5A and B). By contrast, addition of 100 μM 6-BNZ-cAMP (a selective PKA agonist) exerted little effects (1.26-fold increase, $P > 0.05$), on phosphorylated syntabulin level (Fig. 5A and B). Forskolin, 8-pCPT-2′-*O*-Me-cAMP or 6-BNZ-cAMP did not alter the total syntabulin protein level (Fig. 5A and B). These results suggest that syntabulin may involve in cAMP-Epac2 signaling pathway.

We next determined the role of syntabulin in cAMP-Epac2-enhanced glucose-stimulated insulin secretion in INS-1E cells. As shown in Fig. 5C, specific knockdown of syntabulin diminished the potency of forskolin, 6-BNZ or 8-CPT-2′-*O*-Me-cAMP on glucose-stimulated insulin secretion by 51% or 48% respectively, as compared with vector-transfected cells ($P < 0.01$). Although to a less extent compared to the cases of forskolin and

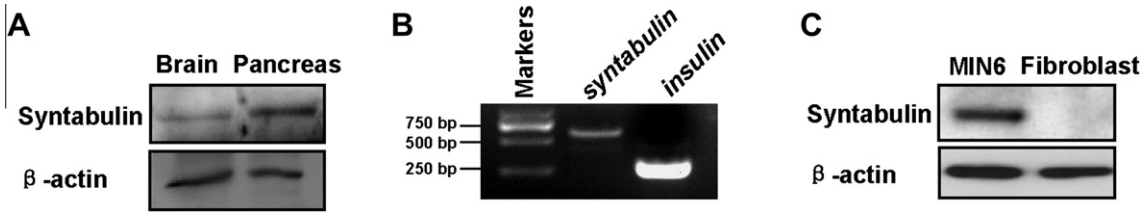

Fig. 1. Syntabulin expression in mouse pancreas and insulin-secreting β-cell lines. (A) Identification of syntabulin protein expression in mouse brain and pancreas by Western blot. β-Actin was used as an internal control. (B) Syntabulin mRNA expression in INS-1E cells was determined by RT-PCR with insulin as an internal control. (C) Syntabulin protein expression in MIN6 cells was confirmed by Western blot with β-actin as an internal control. Fibroblast was used as a negative control for syntabulin expression.

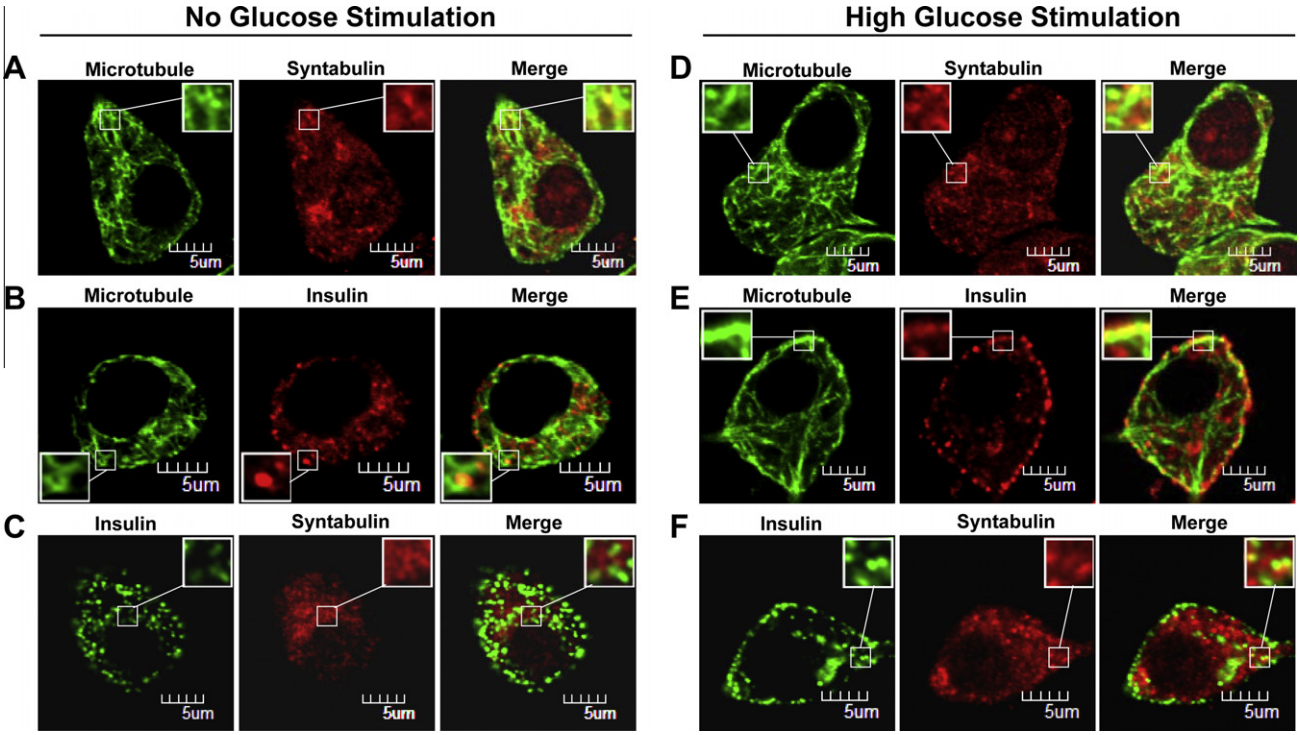

Fig. 2. Co-localization of syntabulin, microtubule and insulin-containing granules in INS-1E cells by indirect immunofluorescence double staining. Distribution of microtubule (green, left) and syntabulin (red, middle) and co-localization of the two (yellow, right) at basal condition (no glucose) (A) and stimulation with 16.8 mM glucose for 10 min (D); microtubule (green, left) and insulin (red, middle) and co-localization of the two (yellow, right) at basal condition (B) and stimulation with 16.8 mM glucose for 10 min (E); insulin (green, left) and syntabulin (red, middle) and co-localization of the two (yellow, right) at basal condition (C) and stimulation with 16.8 mM glucose for 10 min (F) in INS-1E cells. Scale bars represent 5 μm.

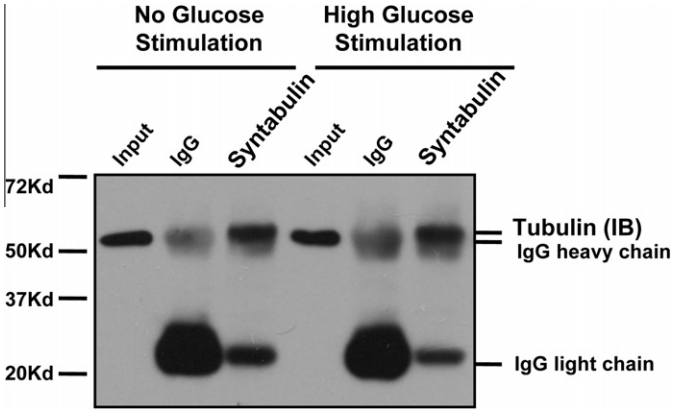

Fig. 3. Interaction of syntabulin with microtubule in INS-1E cells determined by immunoprecipitation analysis. INS-1E cells were treated without or with 16.8 mM glucose for 10 min, followed by immunoprecipitation with syntabulin antibody and immunoblotting with antibody against microtubule. The faint bands shown in the IgG lanes with almost the same size as microtubule are the IgG heavy chain.

8-CPT-2′-*O*-Me-cAMP, down-regulation of syntabulin diminished the potency of 6-BNZ on insulin secretion by 44% ($P < 0.01$). Thus syntabulin is required for cAMP-linked-enhancement of glucose-stimulated insulin secretion.

4. Discussion

Syntabulin is a Kif5b adaptor protein found in a variety of tissues [16] and has been demonstrated to play a critical role in anterograde transport of presynaptic cargoes including secretory vesicles in neurons [16,23]. In this study we show that syntabulin is also expressed in mouse pancreas (Fig. 1A) as well as in clonal β-cell lines (Fig. 1B and C). Moreover, syntabulin was largely present in the cytoplasm of INS-1E cells and resided in multiple granulated foci (Fig. 2A, C, D and F). Different from its distribution found in neurons and COS-7 cells where syntabulin was found to distribute along microtubules and associate with vesicles or vesicular-tubular structures [16], a few of co-localizations of syntabulin with microtubule (Fig. 2A; Suppl. Fig. 2) or insulin-containing granules (Fig. 2C; Suppl. Fig. 2) were observed at no glucose conditions. By contrast, the association of syntabulin with microtubules (Fig. 2D; Suppl. Fig. 3) and insulin-containing granules was increased in punctate structures within the cytoplasm of INS-1E cells (Fig. 2F; Suppl. Fig. 3) upon glucose stimulation. Association of syntabulin and microtubule was also supported by the data of immunoprecipitation assay (Fig. 3). Quantitative analysis revealed that co-localization of syntabulin and microtubule was increased by 7% at high glucose (Suppl. Table 1), whereas an increase might not be large enough to be detected by immunoprecipitation analysis (Fig. 3). Nonetheless, these results suggest that the association of syntabulin with microtubule and insulin-containing granules is regulated by glucose.

It is well known that recruitment of insulin-containing granules to the cell surface is essential for the both rapid and sustained insulin secretion in response to glucose [8,24], a process that is mediated by microtubule. Disruption of the microtubule network impairs both the rapid and sustained component of exocytosis of insulin-containing granules (monitored as depolarization-evoked capacitance increases) by 50% [25]. To explore the functional importance of granule-associated syntabulin, we suppressed syntabulin expression in INS-1E cells with syntabulin-targeted shRNA. This molecular approach results in a 45% decrease in syntabulin protein level (Fig. 4A and B) and concomitant significantly diminished glucose-stimulated insulin secretion (by ~40%) as well as basal insulin secretion (by ~10%). These effects on insulin secretion

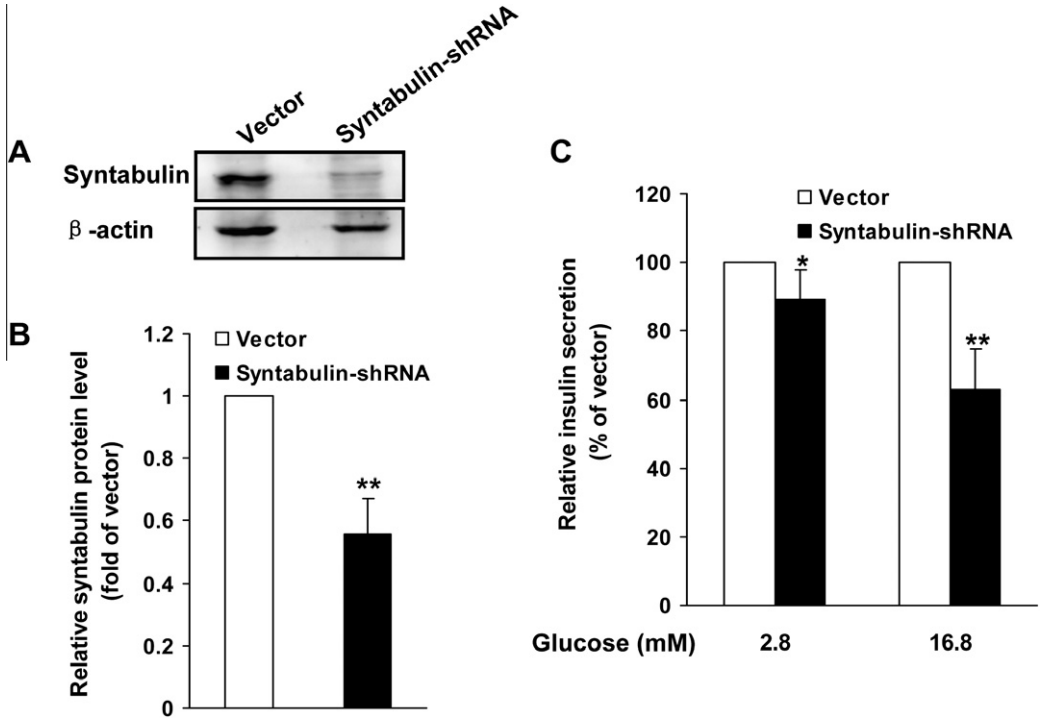

Fig. 4. Effects of knock-down of syntabulin using shRNA on insulin secretion in INS-1E cells. (A) INS-1E cells were transiently transfected with pAd-syntabulin-shRNA (Syntabulin-shRNA) or empty vector (vector). Expression of syntabulin was determined by Western blot using an antibody against syntabulin. β-actin was used as an internal control. (B) Intensities of syntabulin protein expression were quantified, normalized against the level of β-actin and expressed as fold of protein abundance in INS-1E cells transfected with vector control. Data are means ± S.E.M of six independent experiments in duplicate. $^{**}P < 0.01$ vs. vector control. (C) Cells transfected with syntabulin-shRNA or empty vector were first preincubated for 1 h in KRB and then stimulated with 2.8 mM glucose or 16.8 mM glucose for 1 h. Insulin secretion was measured by ELISA. Insulin levels (ng/ml) were normalized against total cellular protein content in mg and expressed as percentage change as compared with vector control. Data are means ± S.E.M of six independent experiments in duplicate. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. vector control.

are expected given that the association of syntabulin with insulin-containing granules was more evident during glucose stimulation (Fig. 2F and Suppl. Fig. 1C), whereas rare at no glucose (Fig. 2C). Consistently it has been reported that the numbers of microtubule-based rapid granule movements were increased at high glucose [7]. Therefore syntabulin is essential for the maintenance of insulin secretion, particularly of glucose-stimulated insulin secretion.

It has been proposed that the effects of glucose on microtubule-based granule movements are mediated by the motor protein kinesin. Transfection of INS-1 and MIN-6 cells with a dominant negative kinesin heavy chain mutant abolished rapid granule movements and sustained insulin secretion [8]. Kif5b as a membrane of kinesin superfamily proteins expressed in primary mouse pancreatic β-cells, and suppression of Kif5b with antisense oligonucleotides blunted glucose-stimulated insulin secretion [15], due to the severely slowed vesicle mobilization and decreased rate of RRP replenishment [23]. We now extend these observations and demonstrate that the maintenance of the secretory capacity of pancreatic β-cells also requires the Kif5b adaptor protein syntabulin. This notion is further supported by the observation that the adaptor syntabulin conjoins kinesin and syntaxin-1 in forming the complex of kinesin-syntabulin-syntaxin-1 that plays an essential role in synaptic vesicle anterograde transport [17].

The ability of cAMP-increasing agents to stimulate insulin secretion is well established. cAMP action in insulin secretion is known to be mediated through PKA and Epac2 [21,26]. Epac2 is a newly recognized family of cAMP-binding proteins and mediates cAMP-regulated exocytosis of insulin-containing granules [21,26]. Here we demonstrate that stimulation of Epac2 by forskolin or 8-pCPT-2′-*O*-Me-cAMP significantly increases phosphorylated syntabulin level; whereas activation of PKA has little effects (Fig. 5A and B). The precise molecular mechanisms underlying phosphorylation of syntabulin by Epac2 are not clear. It is known that subcellular localization of Epacs is important for activation of downstream signaling. For example targeting of Epac to perinuclear region is required for cAMP-mediated protein kinase B activation in HEK cells [27]. It requires further studies to confirm that stimulation of Epac2 involves in activation of PKB, and subsequently results in the phosphorylation of syntabulin in pancreatic β-cells.

We also show that syntabulin is required for cAMP-Epac2 and cAMP-PKA enhanced glucose stimulated insulin secretion (Fig. 5C). Involvement of syntabulin in cAMP-Epac2 stimulated insulin secretion would be understandable in terms of the observations that microtubule-associated protein (MAP) 1A is a protein-binding partner for Epac1 and Epac2 via interaction of its light chain with the catalytic domain of Epac [28]. Thus syntabulin could be a novel effector of Epac2 and involve in cAMP-Epac2-regulated insulin secretion in pancreatic β-cells. On the contrary, it is perhaps unexpected to find that syntabulin also involves in cAMP-PKA-enhanced insulin secretion (Fig. 5C), given that the PKA agonist 6-BNZ has little effects on phosphorylation of syntabulin (Fig. 5A and B). These observations are not necessarily contradictory because it has been found that PKA regulates microtubule-based granule transport via phosphorylation of kinesin [29], a component of the complex of kinesin-syntabulin-syntaxin-1 [17]. Thus knock-down of syntabulin or disruption of the complex would ultimately impair PKA-regulated granule transport and secretion.

In summary, we demonstrate in this study that syntabulin as a new microtubule associated protein involves in glucose-stimulated insulin secretion. Importantly syntabulin may act as a linker molecule connecting cAMP signaling and microtubule-based granule transport, and play a critical role in cAMP-potentiated insulin secretion.

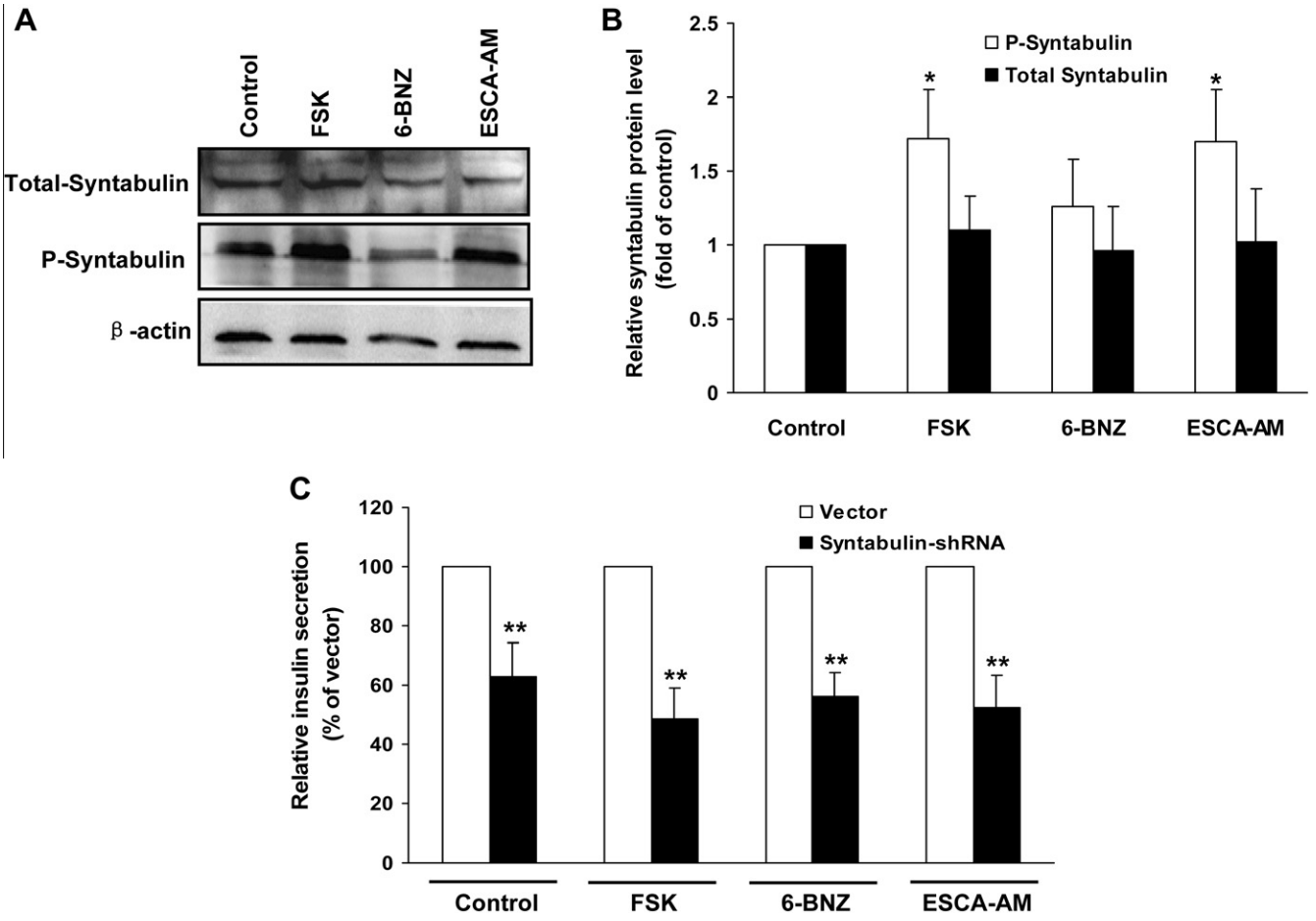

Fig. 5. Syntabulin is involved in cAMP-Epac2-potentiated glucose-stimulated insulin secretion in INS-1E cells. (A) INS-1E cells were stimulated by 16.8 mM glucose alone (control), addition of 10 μM foskolin (FSK), 100 μM 6-BNZ-cAMP-AM (6-BNZ), or 1 μM 8-pCPT-2′-*O*-Me-cAMP (ESCA-AM) for 15 min, respectively. Expression of total syntabulin and phosphorylated-syntabulin (P-Syntabulin) under various stimulations was determined by Western blot using antibodies against total syntabulin and P-Syntabulin, respectively. β-actin was used as an internal control. Pictures shown are representative experiments of four independent experiments. (B) Intensities of total and phosphorylated syntabulin in cells upon stimulation were quantified, normalized against the level of β-actin and expressed as fold change of glucose stimulation alone (control). Data are means ± S.E.M of four independent experiments. $^{*}P < 0.05$ vs. control. (C) Cells transfected with syntabulin-shRNA or empty vector were stimulated by 16.8 mM glucose alone (control), addition of 10 μM FSK, 100 μM 6-BNZ, or 1 μM ESCA-AM for 30 min, respectively. Insulin secretion level (ng/ml) was normalized against total cellular protein content in mg and expressed as percentage of empty vector. Data are means ± S.E.M of six independent experiments in duplicate. $^{**}P < 0.01$ vs. empty vector.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2012.08.025.

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